

1 **Temporal progression of photosynthetic-strategy in**
2 **phytoplankton in the Ross Sea, Antarctica**

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25 **Abstract**

26

27 The bioavailability of iron influences the distribution, biomass and productivity of
28 phytoplankton in the Ross Sea, one of the most productive regions in the Southern
29 Ocean. We mapped the spatial and temporal extent and severity of iron-limitation of
30 the native phytoplankton assemblage using long- (>24 h) and short-term (24 h) iron-
31 addition experiments along with physiological and molecular characterisations during
32 a cruise to the Ross Sea in December-February 2012. Phytoplankton increased their
33 photosynthetic efficiency in response to iron addition, suggesting proximal iron
34 limitation throughout most of the Ross Sea during summer. Molecular and
35 physiological data further indicate that as nitrate is removed from the surface ocean the
36 phytoplankton community transitions to one displaying an iron-efficient photosynthetic
37 strategy characterised by an increase in the size of photosystem II (PSII) photochemical
38 cross section (σ_{PSII}) and a decrease in the chlorophyll-normalised PSII abundance.
39 These results suggest that phytoplankton with the ability to reduce their photosynthetic
40 iron requirements are selected as the growing season progresses, which may drive the
41 well-documented progression from *Phaeocystis antarctica*- assemblages to diatom-
42 dominated phytoplankton. Such a shift in the assemblage-level photosynthetic strategy
43 potentially mediates further drawdown of nitrate following the development of iron
44 deficient conditions in the Ross Sea.

45

46 **Keywords**

47 Iron, Phytoplankton, Photosynthetic proteins, Photosystem II, Nutrient limitation,

48 Ross Sea

49

50 **Highlights**

51

- 52 • Phytoplankton in the Ross Sea change their photosynthetic physiology over
53 the growing season to a strategy requiring less iron.
- 54 • This results in fluorescence yields per chlorophyll and PSII both increasing as
55 the growing season develops.
- 56 • This observation may help explain the well characterised seasonal progression
57 from to *Phaeocystis* spp. to diatom spp. over the growing season and also have
58 implications for the assessment of primary production from estimates of
59 chlorophyll in this region.

60

61

62 **1. Introduction**

63

64 The Ross Sea continental shelf is the most productive region in the Southern
65 Ocean (Arrigo and van Dijken, 2004; Peloquin and Smith, 2007), with an annual
66 productivity $>200 \text{ g C m}^{-2}$ (Smith et al., 2006), which may account for as much as 27%
67 of the estimated total Southern Ocean biological CO_2 uptake (Arrigo et al., 2008). An
68 understanding of the controls on primary productivity is therefore needed given the
69 potential for future changes in stratification (Boyd et al., 2008; Smith et al., 2014) and
70 nutrient inputs to this region (Mahowald and Luo, 2003; Tagliabue et al., 2008).

71 A persistent polynya in the southern Ross Sea greatly increases in size in the
72 early austral spring (Arrigo and van Dijken, 2003; Reddy et al., 2007), and hosts large
73 seasonal phytoplankton blooms, typically dominated by the colonial haptophyte
74 *Phaeocystis antarctica* (*P. antarctica*) in spring through early summer (November –
75 December), with an increase in abundance of diatoms in mid- to late summer (Arrigo
76 and van Dijken, 2004; Arrigo et al., 1998; DiTullio and Smith, 1996; Goffart et al.,
77 2000; Smith and Gordon, 1997; Smith et al., 2000). Understanding the causes and
78 consequences of this seasonal phytoplankton progression is important, as the spatial
79 and temporal distribution and abundance of *P. antarctica* and diatoms have significant
80 biogeochemical consequences on, for example, the elemental composition and flux of
81 biogenic material from the euphotic zone (Arrigo et al., 1999; DeMaster et al., 1992;
82 Smith and Dunbar, 1998; Tagliabue and Arrigo, 2005).

83 Iron (Fe) and irradiance are assumed to exert the major ‘bottom-up’ controls on
84 phytoplankton biogeography and productivity in the Ross Sea, given the incomplete
85 macronutrient removal at the end of the growing season (Arrigo and van Dijken, 2003;
86 Arrigo et al., 1998; Coale et al., 2003; Fitzwater et al., 2000; Sedwick et al., 2000;
87 Sedwick et al., 2007; Smith et al., 2003; Smith et al., 2000; Tagliabue and Arrigo,
88 2003). Light availability may limit spring phytoplankton growth when vertical mixing
89 is deep and daily integrated irradiance is low, this mixing will also supply dissolved
90 iron (DFe) to the euphotic zone (McGillicuddy et al., 2015). As the growing season
91 progresses and the water column stratifies, the flux of DFe from below is likely reduced
92 and may therefore become a more significant factor in limiting phytoplankton growth
93 rates. Indeed, shipboard iron-addition experiments have repeatedly demonstrated the

94 role of iron limitation in the Ross Sea (Bertrand et al., 2007; Coale et al., 2003; Cochlan
95 et al., 2002; Martin et al., 1990; Olson et al., 2000; Sedwick and DiTullio, 1997;
96 Sedwick et al., 2000), consistent with other metrics of Fe stress including high levels
97 of flavodoxin (Maucher and DiTullio, 2003) and enhanced biological drawdown of
98 silicate relative to nitrate (Arrigo et al., 2000; Smith et al., 2006).

99 Changes in phytoplankton composition from *P. antarctica* to diatom species
100 may be linked to the co-limitation and interaction between iron and light. Boyd (2002)
101 speculated that *P. antarctica* growth is limited by Fe availability from spring through
102 late summer. Sedwick et al. (2007) further proposed that decreases in iron availability
103 through spring are mitigated by increases in irradiance, thereby decreasing
104 phytoplankton iron requirements. The differences in intracellular iron requirements
105 alongside changes in the light environment may explain the community succession of
106 the Ross Sea, where diatoms can outcompete *P. antarctica* in the late summer (Strzepek
107 et al., 2012).

108 Phytoplankton that dominate in the Ross Sea may therefore need to be adapted
109 to highly variable iron concentrations and light availability (Sedwick et al., 2011). An
110 antagonistic relationship between irradiance and photosynthetic Fe demand may be
111 predicted given that lower irradiances can increase Fe requirements associated with the
112 synthesis of the additional photosynthetic units required to increase light absorption
113 (Maldonado et al., 1999; Raven, 1990; Sunda and Huntsman, 1997). Each
114 photosynthetic electron transfer chain requires 22-23 Fe atoms, and the photosynthetic
115 apparatus can be the largest sink of Fe within a phytoplankton cell (Raven, 1990; Shi
116 et al., 2007; Strzepek and Harrison, 2004). In contrast to the tight link between cellular
117 Fe requirements and light harvesting capacity, studies on Southern Ocean diatoms and
118 *P. antarctica* in culture suggest the Fe burden of photosynthesis may be significantly
119 reduced for these species through increases in the size rather than the number of
120 photosynthetic units (termed sigma-type acclimation) in response to iron/ and light
121 limitation (Strzepek et al., 2012; Strzepek et al., 2011). Effectively, these Southern
122 Ocean taxa appear to invest relatively more resources in the generation of a larger light-
123 harvesting apparatus, rather than in the Fe-rich photosynthetic catalysts of
124 photosystems I and II (Strzepek et al., 2012). This Fe-efficient strategy appears to be
125 most pronounced for Southern Ocean diatoms, which, in culture can have some of the
126 largest light harvesting antennae reported (Strzepek et al., 2012), a phenotype which is
127 more commonly associated with small cells (Suggett et al., 2009). The photosynthetic

128 strategy of Southern Ocean diatoms may therefore contribute to the apparently low Fe
129 requirement and cellular Fe:C ratio of these species (Coale et al., 2003; Kustka et al.,
130 2015; Sedwick et al., 2007; Strzepek et al., 2012; Strzepek et al., 2011), and as such
131 drive the seasonal progression from *P. antarctica* to diatoms in the Ross Sea.

132 In December-February 2012 a research cruise was conducted as part of the
133 multidisciplinary research project *Processes Regulating Iron Supply at the Mesoscale*
134 *– Ross Sea* (PRISM-RS), in an effort to identify and quantify the major sources of iron
135 to the surface waters of the Ross Sea during the growing season. As part of this study,
136 physiological and molecular measurements were combined with shipboard incubation
137 experiments in an effort to define the spatial and temporal extent of phytoplankton iron
138 limitation and reveal the photosynthetic strategy of the phytoplankton assemblages.

139 2. Materials and Methods

140

141 2.1. Oceanographic Sampling

142

143 The samples and data presented here were obtained during a cruise of the *RVIB*
144 *Nathaniel B. Palmer* to the Ross Sea (cruise NBP12-01) from 24th December 2011 to
145 10th February 2012 (DOY 358 – 041). During the cruise, 29 short-term (24 h) and 3
146 long-term (168 h) incubation experiments were performed (Fig. 1a). Short-term
147 experiments were used to determine rapid iron induced changes in the phytoplankton
148 photophysiological status; whereas long-term experiments determined whether relief
149 from iron limitation could drive changes in biomass. For the long-term incubation
150 experiments, uncontaminated whole seawater was collected from ~5 m depth whilst
151 slowly underway, using a trace-metal clean towed fish system (Sedwick et al., 2011).
152 Uncontaminated whole seawater for the short-term incubation experiments was
153 collected from ~10 m depth in Teflon-lined, external closure 5 L Niskin-X samplers
154 (General Oceanics) deployed on a trace metal clean CTD rosette system (Marsay et al.,
155 2014). Samples for additional analysis were also collected along the cruise track.

156

157 2.2. Bioassay Incubation Experiments

158

159 Incubation experiments were performed using methods similar to those employed
160 previously in the Southern Ocean (Moore et al., 2007; Nielsdóttir et al., 2012) and the
161 high latitude North Atlantic (HLNA) (Nielsdóttir et al., 2009; Ryan-Keogh et al., 2013).
162 Water for the experiments (see 2.1, above) was transferred unscreened into acid-washed
163 1.0-L polycarbonate bottles (Nalgene) for the short-term incubation experiments and
164 4.5-L polycarbonate bottles for the long-term incubation experiments. Incubation
165 bottles were filled in a random order, with triplicate samples for initial measurements
166 in the long-term incubation experiments collected at the beginning, middle and end of
167 the filling process. Initial samples for the short-term incubation experiments were
168 collected from the same Niskin-X sampling bottle. The short-term experiments were
169 run for 24 h and the long-term experiments were run for 168 h; both experiments
170 consisted of two treatments: an unamended control treatment and 2.0 nmol L⁻¹ Fe

171 treatment (hereafter, + Fe). All experimental incubations were conducted as biological
172 duplicates or triplicates.

173 All bottle tops were externally sealed with film (Parafilm™), and bottles were
174 double bagged with clear polyethylene bags to minimize risks of contamination during
175 the incubation. On-deck incubators were shaded using LEE “blue lagoon” filters to
176 provide light levels corresponding to ~35% of above-surface irradiance (Hinz et al.,
177 2012; Nielsdóttir et al., 2009; Ryan-Keogh et al., 2013). Flowing surface seawater was
178 used to control the temperature in the incubators. Subsampling of long-term incubations
179 for measurements of chlorophyll *a*, dissolved macronutrient concentrations and
180 phytoplankton physiological parameters occurred after 24, 72, 120 and 168 h. Sub-
181 sampling of short-term incubation experiments for the same parameters occurred after
182 24 h. All experiments were set up and sub-sampled under a class-100 laminar flow hood
183 within a trace metal clean environment.

184

185 2.3. Chlorophyll *a* and Nutrient Analysis

186

187 Samples for chlorophyll *a* (Chl) analysis (250 mL) were filtered onto GF/F filters and
188 then extracted into 90% acetone for 24 h in the dark at 4°C, followed by analysis with
189 a fluorometer (TD70; Turner Designs) (Welschmeyer, 1994). Macronutrient samples
190 were drawn into 50 mL diluvials and refrigerated at 4°C until analysis, which typically
191 commenced within 12 h of sampling. Nitrate plus nitrite (DIN), phosphate, ammonium
192 and silicate were determined shipboard on a five-channel Lachat Instruments
193 QuikChem FIA+ 8000s series AutoAnalyser (Armstrong et al., 1967; Atlas et al., 1971;
194 Bernhardt and Wilhelms, 1967; Patton, 1983). Dissolved iron was determined post-
195 cruise using flow injection analysis modified from Measures et al. (1995), as described
196 by Sedwick et al. (2011); accuracy of the DFe method was verified by analysis of SAFe
197 reference seawater samples (Johnson et al., 2007).

198

199 2.4. Phytoplankton Photosynthetic Physiology

200

201 Variable chlorophyll fluorescence was measured using a Chelsea Scientific Instruments
202 Fastracka™ Mk II Fast Repetition Rate fluorometer (FRRf) integrated with a FastAct™
203 Laboratory system. All samples were acclimated in opaque bottles for 30 minutes at *in*
204 *situ* temperatures, and FRRf measurements were blank corrected effect using carefully

205 prepared 0.2 μm filtrates for all samples (Cullen and Davis, 2003). Blanks were
206 typically around 1% and always <10% of the maximum fluorescence signal. Protocols
207 for FRRf measurements and data processing were similar to those detailed elsewhere
208 (Moore et al., 2007). Data from the FRRf were analysed to derive values of the
209 minimum and maximum fluorescence (F_o and F_m) and hence F_v/F_m (where $F_v = F_m -$
210 F_o), as well as the functional absorption cross-section of PSII (σ_{PSII}) by fitting transients
211 to the model of Kolber et al. (1998).

212

213 2.5. Phytoplankton Composition

214

215 Samples for photosynthetic pigment analysis were collected and measured by high
216 performance liquid chromatography (HPLC). 0.3 – 1.0 L of sea-water were filtered
217 through GF/F filters, which were immediately flash frozen in liquid nitrogen and stored
218 at -80°C until analysis. Pigments were extracted into 90% acetone by sonification
219 before quantification using a Waters Spherisorb ODSU C-18 HPLC column and Waters
220 HPLC system as described in Smith et al. (2006). Algal community composition was
221 then estimated from pigment concentrations following the method of Arrigo et al.
222 (1999).

223

224 2.6. Total Protein Extraction and Quantification

225

226 Photosynthetic protein abundances were quantified using techniques similar to those
227 described elsewhere (Brown et al., 2008; Macey et al., 2014; Ryan-Keogh et al., 2012).
228 Samples for protein extraction were collected by filtering 1.0-3.0 L of seawater onto
229 GF/F filters (Whatman) under low light for ~45 minutes to minimize changes in protein
230 abundance following sampling. Filters were flash frozen and stored at -80°C until
231 analysis. Proteins were extracted in the laboratory according to the protocol described
232 by Brown et al. (2008). Quantification was performed using custom Agrisera™ primary
233 antibodies and peptide standards, which were designed against peptide tags conserved
234 across all oxygenic photosynthetic species for protein subunits that are representative
235 of the functional photosynthetic complex PsbA (PSII) (Campbell et al., 2003). Protein
236 abundances were quantified using QuantityOne™ and ImageLab™ software;
237 quantification was performed within the unsaturated portion of the calibration curve.
238 The estimated protein abundances were comparable to those reported for natural

239 phytoplankton communities using similar methods (Hopkinson et al., 2010; Losh et al.,
240 2013; Macey et al., 2014; Richier et al., 2012).
241

242 **3. Results and Discussion**

243

244 3.1. General Oceanography

245

246 A range of oceanographically distinct regions was occupied on the Ross Sea continental
247 shelf during the PRISM-RS cruise (Fig. 1). These included areas close to the Ross Ice
248 Shelf, near and within pack ice, and over shallow bathymetric features, both of which
249 may provide important sources of DFe to the upper water column (McGillicuddy et al.,
250 2015). Highest chlorophyll a concentrations (Fig. 2a) were associated with the ice-shelf
251 in the southwestern Ross Sea ($24.6 \mu\text{g Chl L}^{-1}$) and correlated with the lowest DIN
252 (dissolved inorganic nitrate + nitrite) concentrations (Figs. 2b, 3) and lowest surface
253 F_v/F_m values observed (Figs. 2c, 3). Surface DFe concentrations ranged from 0.067-
254 0.787 nM (Fig. 2d), were not correlated with chlorophyll or DIN concentrations (Fig.
255 3, Supplementary Information, Fig. S1), and were elevated off the continental shelf in
256 the northeast sector of the Ross Sea.

257

258 3.2. Mapping of Iron Limitation

259

260 Despite being the most productive region in the Southern Ocean, our results confirm
261 that phytoplankton growth in the Ross Sea is limited by iron availability during
262 summer, consistent with previous studies (Bertrand et al., 2011; Bertrand et al., 2007;
263 Coale et al., 2003; Cochlan et al., 2002; Martin et al., 1990; Olson et al., 2000; Sedwick
264 and DiTullio, 1997; Sedwick et al., 2000). The response of phytoplankton to iron-
265 addition was assayed through a series of long- (168 h) and short-term (24 h) iron-
266 addition incubations (Fig. 1), while no clear spatial pattern in iron stress could be
267 observed from a single cruise during a time of relatively rapid changes in a spatio-
268 temporally complex system (Fig. 2), there was evidence of an increase in
269 photosynthetic efficiency following iron addition throughout much of the Ross Sea
270 during summer, highlighting the role of iron in influencing phytoplankton physiology.
271 To compare these iron-mediated changes in F_v/F_m , $\Delta(F_v/F_m)$ was calculated as defined
272 in Ryan-Keogh et al. (2013), as the difference between the Fe-amended and control
273 treatments (Equation 1).

274

275 Equation 1 Calculation of $\Delta(F_v/F_m)$.

$$276 \quad \Delta(F_v/F_m) = \frac{F_v/F_{m+Fe} - F_v/F_{mControl}}{Time}$$

277 Values of $\Delta(F_v/F_m)$ were frequently positive following iron addition (ranging from 0.00
278 - 0.17) (Fig. 4a), suggesting that Fe amendments increased the photosynthetic
279 efficiency of phytoplankton in much of the Ross Sea during the sampling period.

280 Data from long-term (168 h) experiments (Table 1 and Fig. 4) enable a more
281 detailed analysis of the response of phytoplankton to iron-additions. Three experiments
282 were initiated from (1) near the Ross Ice Shelf, (2) over the Ross Bank and (3) in an
283 anti-cyclonic eddy (Figs. 1 and 4). The three experiments revealed varying responses
284 to iron additions by the extant phytoplankton assemblage. Experiments 1 and 3 gave a
285 strong and positive response to iron additions, and provided evidence that
286 phytoplankton were iron limited. Shorter-term responses revealed elevated values of
287 F_v/F_m (i.e., a positive $\Delta(F_v/F_m)$) after 24 h (Fig. 4a), with subsequent significant
288 (ANOVA, $p < 0.05$) increases in growth rates and nutrient removal observed after 168 h
289 (Table 1). Experiment 2, initiated over the Ross Bank, did not show an increase in
290 photosynthetic efficiency $\Delta(F_v/F_m)$ (Fig. 4a). Moreover, growth rate and nutrient
291 removal were not significantly different between control and iron-addition conditions
292 until after >168 h (ANOVA, $p > 0.05$) (Table 1), which most likely reflects severe
293 depletion of ambient DFe in the control treatments by this time. The Ross Bank (Fig.
294 4a, Table 1) has a shallow bathymetry (~ 150 m), and none of the Fe-addition
295 experiments in this region showed a significant response (Fig. 4). The Ross Bank may
296 therefore provide significant and continuous DFe inputs to the euphotic zone, thereby
297 ultimately stimulating productivity.

298 The measurement of F_v/F_m is derived from analysis of the fluorescence kinetics
299 emitted from the photosynthetic reaction centre photosystem II (PSII) and its associated
300 light-harvesting antenna (Kolber and Falkowski, 1993). Understanding the mechanism
301 of changes to F_v/F_m can provide information on the process by which phytoplankton
302 respond to iron-limitation. Absolute changes in maximum fluorescence (F_m) and
303 variable fluorescence (F_v) normalised to chlorophyll *a* were calculated (Figs. 4b and c),
304 revealing a significant difference between the +Fe and control treatments in $F_m \text{ Chl}^{-1}$ (t
305 = 24 h (t -test, $p < 0.05$)), whereas there was no significant difference for $F_v \text{ Chl}^{-1}$ (t = 24
306 h (t -test, $p > 0.05$)). This suggests that changes in F_v/F_m reflect changes in the proportion
307 of chlorophyll that is photosynthetically coupled to active PSII reaction centres, rather

308 than changes in the activity of PSII (Behrenfeld et al., 2006; Lin et al., 2016; Macey et
309 al., 2014). A similar response was observed for all short-term iron-addition
310 experiments that exhibited positive changes in $\Delta(F_v/F_m)$.

311

312 3.3 Temporal Development of Photosynthetic Strategy

313

314 Given the high degree of spatial variability in response to iron-additions, we placed all
315 observations within a unified framework, hence producing a conceptualised model of
316 temporal progression of phytoplankton within the Ross Sea. The PRISM-RS cruise
317 sampled for 30 days covering a period from mid- to late summer, during which we
318 expected iron limitation of phytoplankton growth to be significant (Sedwick et al.,
319 2000). Total phytoplankton biomass accumulation is dependent on growth after the
320 sampled regions become ice-free (Arrigo and van Dijken, 2003) and the losses due to
321 grazing, sinking and physical removal. All spatial data therefore represent a mosaic of
322 different temporal progressions that represent different stages of phytoplankton
323 development. We utilise surface nitrate (DIN) as a proxy to separate the temporal
324 patterns from any spatial differences (Fig. 5). As phytoplankton biomass (Chl)
325 increased, nutrients were removed and F_v/F_m reduced (Figs. 3, 5a). Pigment data
326 showed that the nutrient drawdown and Chl increase in parallel with a shift from *P.*
327 *antarctica*-dominated to diatom-dominated assemblages (Figs. 3, 5b). Within this
328 conceptual framework, the relative severity of Fe-stress ($\Delta F_v/F_m$) may be inferred from
329 the Fe-addition incubation experiments. Two potential phases of Fe deficiency were
330 identified (Fig. 5c): first, when DIN concentrations remain high ($> \sim 20 \mu\text{M}$) and *P.*
331 *antarctica* is a major component of the phytoplankton (labelled '1'), and secondly when
332 DIN is further removed (to $< \sim 20 \mu\text{M}$) by diatom-dominated communities (labelled '2';
333 Fig. 5c).

334 Photophysiological parameters are presented within this framework. The
335 relative size of the effective light-harvesting cross-section of PSII (σ_{PSII}) (Fig. 6a) is
336 low ($\sim 1.6 \text{ nm}^2$) when DIN and F_v/F_m are high, and approximately doubles to $\sim 3.29 \text{ nm}^2$
337 as DIN is depleted and the assemblage becomes diatom-dominated. Quantification of
338 the photosynthetic catalyst PSII further characterises the photosynthetic strategy of
339 phytoplankton in the Ross Sea. Chlorophyll normalised to abundances of the protein
340 target PsbA (indicative of the abundance of PSII; (Brown et al., 2008) (Chl:PsbA),
341 which can provide another indication of the relative sizes of the light harvesting

342 pigment antenna relative the abundance of the photosystems, is lower at higher DIN
343 concentrations and increases as DIN and F_v/F_m decrease (Fig. 6b). Combining the
344 protein abundance data and the photophysiological measurements, the maximum
345 fluorescent yield per chlorophyll ($F_m:Chl$) (Fig. 6c) and per PSII ($F_m:PsbA$) (Fig. 6d)
346 can also be calculated. Both of these parameters increase, by 46 and 296% respectively,
347 with decreases in DIN and F_v/F_m .

348 Together, these photophysiological measurements and corresponding
349 environmental information at the time of sampling therefore indicate several significant
350 correlations (Fig. 3 & Supplementary Information, S1) associated with the potential
351 drivers of the observed transition in community structure and subsequent changes in
352 photophysiology. Thus, within our conceptual frame work, using DIN concentration as
353 a proxy for the stage of the phytoplankton bloom, we observe statistically significant
354 positive correlations ($p<0.01$) with other macronutrients and the photosynthetic
355 efficiency (F_v/F_m) which all decline as nitrate is removed from the system. While
356 negative correlations ($p<0.01$) are observed between DIN and temperature, chlorophyll
357 concentration, the relative abundance of diatoms and σ_{PSII} which all increase as nitrate
358 is removed from the system. While no significant correlation is seen between DIN and
359 the fluorescence yield per PSII ($F_m:PSII$) or the chlorophyll content per PSII ($Chl:PSII$),
360 there is a significant negative correlation between $F_m:Chl$ and $PSII:Chl$ ($p<0.01$) (Fig.
361 3).

362 No statistically significant ($p<0.01$) relationships were observed with dissolved iron
363 concentrations, suggesting that this variable may not represent a good indicator of iron
364 stress, as might be expected considering that any limiting nutrient would be expected
365 to be severely depleted by biological uptake. Overall, the observed correlations are thus
366 taken to be indicative of the phytoplankton community transitioning between dominant
367 groups as SST increases, non-limiting macronutrients are drawn down and the
368 community biomass increases, potential as a result of different Fe utilisation capacities
369 between diatoms and *P. antarctica* (Strzepek et al., 2012). These observations may also
370 support that the hypothesis that Southern Ocean diatoms may both acquire (Kustka et
371 al., 2015) and utilise (Strzepek et al., 2012) iron more effectively than *P. antarctica* and
372 that the community transition may enable further drawdown of nitrate.

373 While there can be an array of reasons for diatoms being better at acquiring and
374 utilising available DFe as it becomes limiting during summer in the Ross Sea,
375 differences in photosynthetic strategy have the potential to be a significant factor in

376 regulating the temporal changes that occur, given that the photosynthetic apparatus
377 represents the dominant sink for Fe in a phytoplankton cell (Raven, 1990; Strzepek and
378 Harrison, 2004). The analysis presented here clearly demonstrates that a different
379 photosynthetic strategy is apparent within the phytoplankton community responsible
380 for the initial DIN removal vs. those responsible for the later DIN removal. These
381 observations of photosynthetic strategy are consistent with some of the
382 ecophysiological differences observed within culture-based studies of Southern Ocean
383 phytoplankton (Strzepek et al., 2012). Phytoplankton in the Ross Sea generally display
384 a large, functional light-harvesting cross section for PSII (σ_{PSII}) compared to temperate
385 species (Smith et al., 2011). As has been proposed (Strzepek et al., 2012), this may
386 reflect a strategy by which cells acclimate and/or adapt through increasing the size of
387 photosynthetic units rather than the number of photosynthetic units in a low Fe
388 environment – thus escaping the typical antagonistic relationship between iron-demand
389 and light capture (Sunda and Huntsman, 1997). Our measurements of the abundance
390 of the photosynthetic catalysis PSII were also consistent with such an observation,
391 whereby the increase in the ratio of Chl:PSII mirrors the increase in σ_{PSII} (Fig. 6b).
392 This strategy could significantly reduce the iron-demand normally associated with the
393 photosynthetic apparatus. Phytoplankton that dominate at low DIN have a particularly
394 large σ_{PSII} and have increased Chl:PSII values by 255%, again in agreement with culture
395 studies in which Southern Ocean diatoms have larger σ_{PSII} than *P. antarctica* (Strzepek
396 et al., 2012).

397 We thus suggest that the diatoms that dominate in summer as DIN is removed may
398 represent a refined strategy to reduced iron availability, noting that previous
399 information from temperate taxa and regions (Suggett et al., 2009) would tend to
400 suggest that relatively high functional cross sections would be unlikely in
401 phytoplankton with large cell sizes typical of many Southern Ocean diatoms (Suggett
402 et al., 2009). Large cells with large σ_{PSII} may, however, result in ecophysiological trade-
403 offs, including a tendency for over-excitation of PSII and photodamage, which may
404 require a rapid PSII repair cycle or a requirement for rapidly inducible and significant
405 non-photochemical quenching (Campbell and Tyystjärvi, 2012; Petrou et al., 2010; Wu
406 et al., 2011), possibly suggesting Antarctic diatoms would require novel
407 photoprotective strategies. Despite these potential negative consequences of a large
408 σ_{PSII} , Antarctic diatoms seem to have adopted a phenotypic response underlining the

409 relevance of iron-availability and providing some explanation for the low Fe:C ratios
410 in some of these species (Strzepek et al., 2012).

411 While the observations in this study were restricted to the summer season they
412 do include DIN concentrations similar to those estimated for the winter mixed layer
413 nitrate concentration (McGillicuddy et al., 2015) and so potentially conditions
414 analogous to a broader seasonal progression in phytoplankton composition in the Ross
415 Sea from *P. antarctica* early in the growing season to diatom-dominance later in
416 summer (Smith et al., 2010). The dataset therefore provides indications of potential
417 contributory mechanisms for this seasonal progression, while also reflecting the large
418 degree of spatial heterogeneity in physical and biological processes throughout the
419 growing season in the Ross Sea (Smith and Jones, 2015).

420 The data presented here also provide insights into the mechanism of the iron-
421 stress response of phytoplankton. Increases in F_v/F_m are commonly reported as a
422 response to Fe addition (Boyd et al., 2008; Feng et al., 2010). Results from the
423 experiments and observations show that increases in F_v/F_m in response to Fe addition
424 and elevated F_v/F_m values in regions with modest DIN drawdown result from reduction
425 in the ratio of $F_m:Chl$ (or $F_m:PSII$) rather than changes in $F_v:Chl$. This is in agreement
426 with similar observations from the high latitude North Atlantic and Equatorial Pacific
427 (Behrenfeld et al., 2006; Lin et al., 2016; Macey et al., 2014) regions and implies that
428 low F_v/F_m results from changes in the coupling of light-harvesting chlorophyll-binding
429 proteins to photosynthesis rather than accumulation of damaged photosystems. Such
430 accumulation of non-photosynthetically active chlorophyll-binding proteins in Fe-
431 limited oceanic regions can have consequences on estimates of productivity in these
432 regions (Behrenfeld et al., 2006).

433 **4. Conclusions**

434

435 The current study represents an analysis of the summer photosynthetic strategies of
436 phytoplankton in the Ross Sea and highlights how different iron-efficiency strategies
437 occur in phytoplankton as Fe becomes limiting and irradiance availability becomes
438 maximal. This is important for understanding Fe usage efficiency in the region. The
439 Ross Sea clearly differs from other high latitude regions due to plankton composition,
440 yet iron availability still contributes to reduced growth rates and macronutrient removal.
441 Even though this system is one of the most productive regions in the Southern Ocean,
442 iron availability still exerts a strong control over summer productivity and biomass
443 accumulation, and any changes in future iron supply induced by climate change could
444 have profound effects. Climate-mediated changes to the mixed layer depth and sea-ice
445 cover could change iron limitation strategies and phytoplankton phenology (Boyd et
446 al., 2012), as well as alterations to iron supply from highly variable supply mechanisms
447 such as Australian and local dust inputs (Mackie et al., 2008). The Southern Ocean is
448 predicted to be particularly biogeochemically significant with respect to climate change
449 (Marinov et al., 2006) and is the only iron-limited HNLC region where the cryosphere
450 plays a major role. An understanding of the role of iron limitation in this highly dynamic
451 environment is thus particularly important; particularly as climate mediated variability
452 is expected to increase.

453

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694

695 **Tables and Fig. Legends**

696

697 **Table 1** Locations for long-term experiments conducted during NBP12-01 along with values
 698 of initial F_v/F_m , $\Delta(F_v/F_m)$ (Equation 1), net growth rates estimated from chlorophyll
 699 accumulation (Supplementary Information, Equation S1) and nitrate drawdown
 700 (Supplementary Information, Equation S2) over 168 h. Shown are averages \pm standard errors
 701 ($n = 3$ or 5), * indicate significant differences (Two-way ANOVA, $p < 0.05$) from control.

702

703 **Fig. 1 Composite map of Southern Ocean MODIS chlorophyll *a* for December 2011 –**
 704 **February 2012. Inset:** Long-term (blue dots) and short-term (red dots) experimental locations
 705 conducted on cruise NBP12-01 in the Ross Sea with 250 m bathymetric contours. Surface *in*
 706 *situ* samples were also collected at these locations and at those marked CTD-station (black
 707 dots).

708

709 **Fig. 2** Surface chlorophyll concentrations ($\mu\text{g L}^{-1}$) from CTD stations **(a)**. Surface DIN
 710 concentrations (μM) **(b)**. Surface F_v/F_m **(c)**. Surface DFe concentrations (nM) **(d)**. Chlorophyll,
 711 DIN and F_v/F_m from samples collected at 1-5 m depth, DFe from samples collected at ~ 10 m
 712 depth.

713

714 **Fig. 3** Matrix of Pearson's linear correlation coefficients between the variables measured in the
 715 surface waters of the Ross Sea, including: sea surface temperature (SST), Nitrate (DIN),
 716 Phosphate (PO_4^{3-}), Silicate (Si), community structure (% Diatoms), chlorophyll concentration,
 717 F_v/F_m , σ_{PSII} , $F_m:\text{PsbA}$, $\text{Chl}:\text{PsbA}$, and dissolved iron concentrations (DFe). The strength of the
 718 linear association between each pair of variables is indicated by the colour of the square, with
 719 the negative and positive correlations denoted by '-' and '+' within all squares where
 720 significant ($p < 0.01$).

721

722 **Fig. 4** Spatial distribution of $\Delta(F_v/F_m)$ calculated from Fe addition incubation experiments **(a)**.
 723 $\Delta(F_v/F_m)$ calculated from long-term Fe-addition incubation experiments in the Ross Sea, both
 724 from (1) near the Ross Ice Shelf, (2) over the Ross Bank and (3) within an anti-cyclonic eddy
 725 **(b)**. The change in chlorophyll normalized maximum fluorescence, ($\Delta F_m \text{ Chl}^{-1}$) from the three
 726 long-term Fe addition incubation experiments **(c)**. The change in chlorophyll normalized
 727 variable fluorescence $\Delta(F_v \text{ Chl}^{-1})$ from the three long-term Fe addition incubation experiments

728 (d). Shown are averages with \pm standard errors ($n = 4$ or 5). * represent statistically significant
729 differences (*t-test*, $p < 0.05$).

730

731 **Fig. 5** Relationship of DIN (μM) and photosynthetic efficiency (F_v/F_m) throughout the Ross
732 Sea as a function of a) chlorophyll concentrations ($\mu\text{g L}^{-1}$), b) phytoplankton composition (%),
733 and c) the relative degree of Fe stress $\Delta(F_v/F_m)$ (c). Grey dots represent stations where DIN and
734 F_v/F_m were measured but no corresponding additional variables were measured.

735

736 **Fig. 6** Relationship of DIN (μM) and photosynthetic efficiency (F_v/F_m) throughout the Ross
737 Sea as a function of a) functional cross-section of photosystem II (σ_{PSII}) (nm^{-2}), b) the ratio of
738 chlorophyll to PsbA (a core subunit of PSII) (mmol mol^{-1}), c) the ratio of the maximum
739 fluorescence yield to chlorophyll ($F_m:\text{Chl}$), and d) the ratio of the maximum fluorescence yield
740 to PsbA ($F_m:\text{PsbA}$). Grey dots represent stations where DIN and F_v/F_m were measured but no
741 corresponding additional variables were measured.

742

743 Table 1

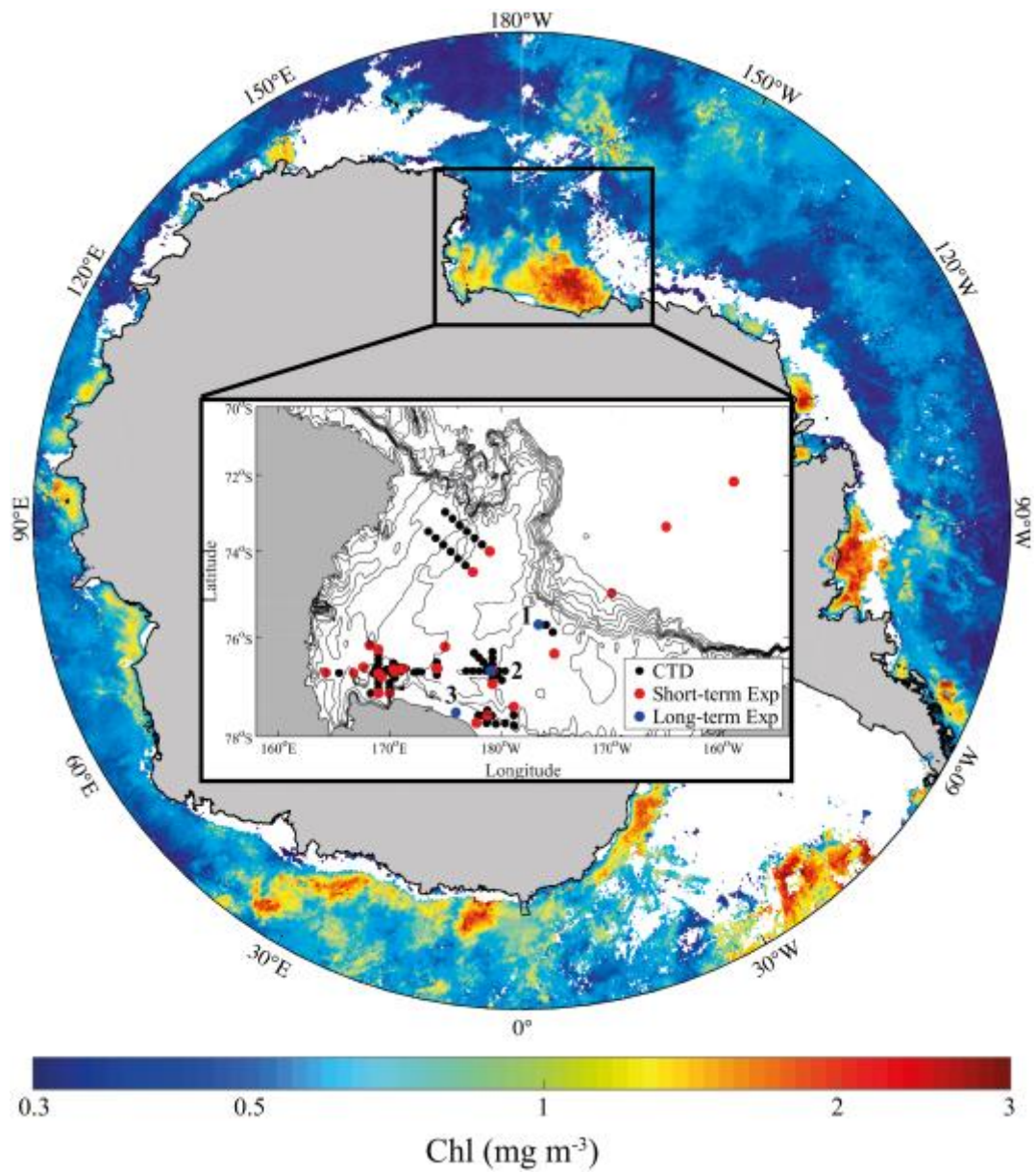
744

Experiment	1	2	3
Lat (°S)	75.72	76.72	77.55
Long (°W)	183.40	179.08	175.97
F_v/F_m Initial	0.26 ±0.01	0.29 ±0.00	0.21 ±0.00
Δ (F_v/F_m), 24 h	0.04 ±0.01	0.00 ±0.00	0.01 ±0.00
μ^{chl}_{Control} (d⁻¹), 0 -168 h	0.11 ±0.02	0.25 ±0.01	0.13 ±0.01
μ^{chl}_{Fe} (d⁻¹), 0 -168 h	0.17* ±0.02	0.29* ±0.00	0.19* ±0.01
ΔNO₃⁻ Control (μ M d⁻¹), 0 – 168 h	1.61 ±0.33	1.50 ±0.04	2.43 ±0.08
ΔNO₃⁻ Fe (μ M d⁻¹), 0 – 168 h	2.53* ±0.13	1.57 ±0.05	2.93* ±0.07

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Fig. 1

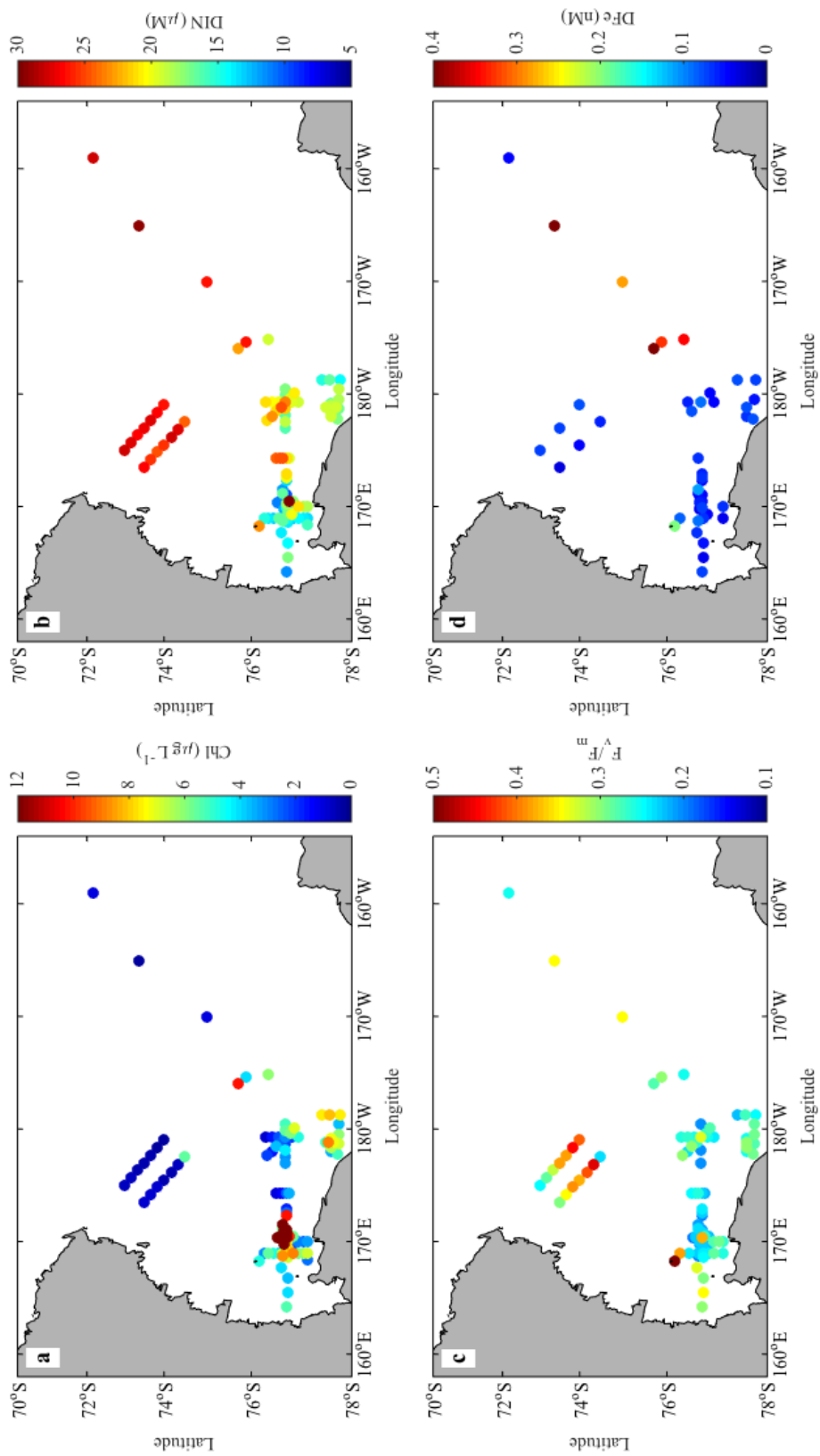


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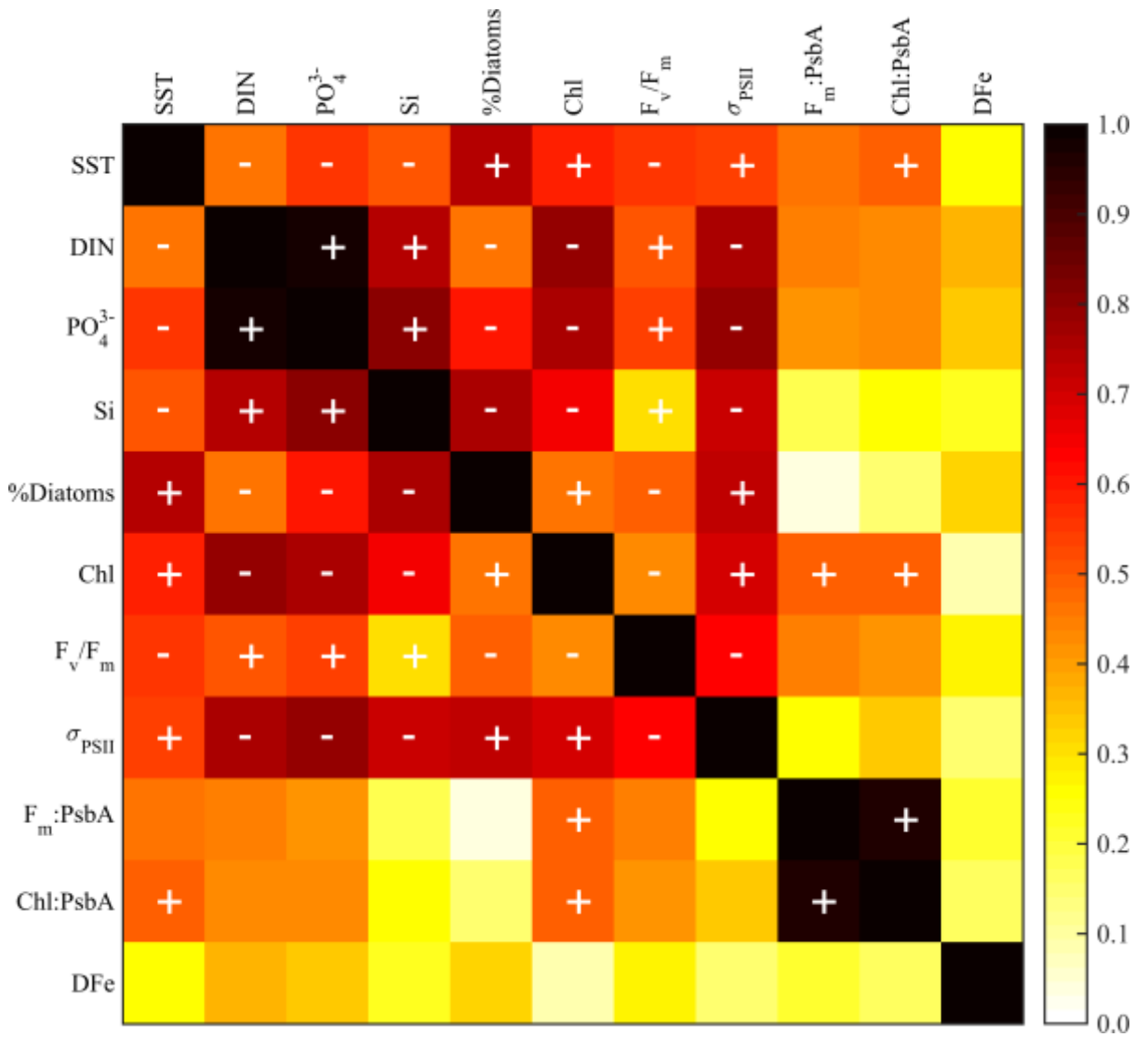
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Fig. 2



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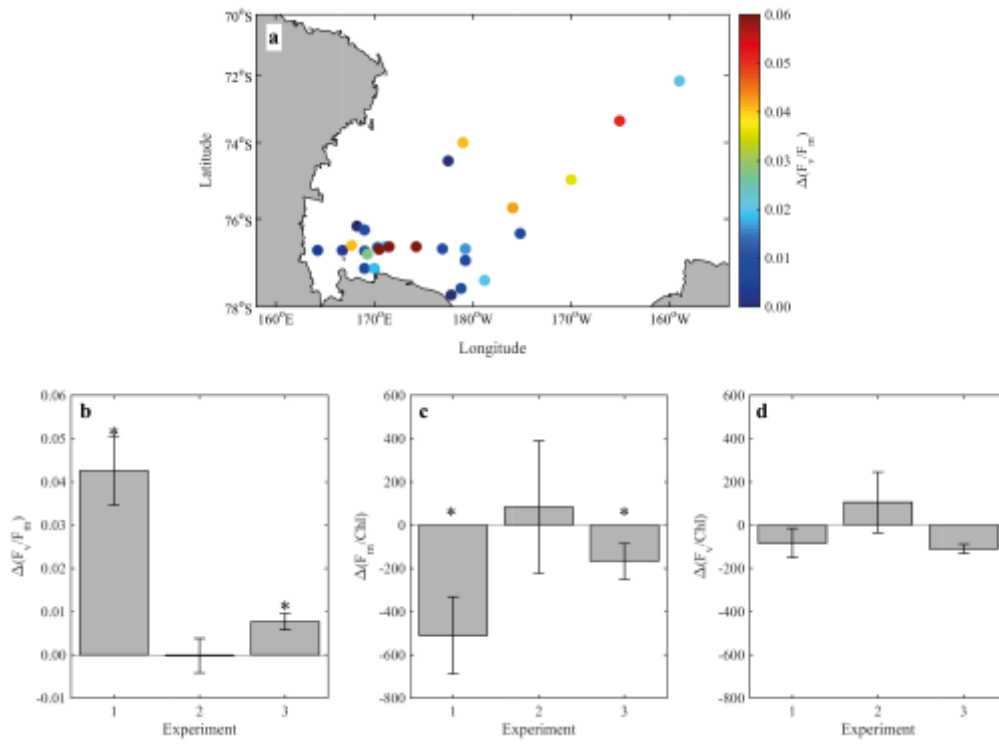
Fig. 3



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755 Fig. 4

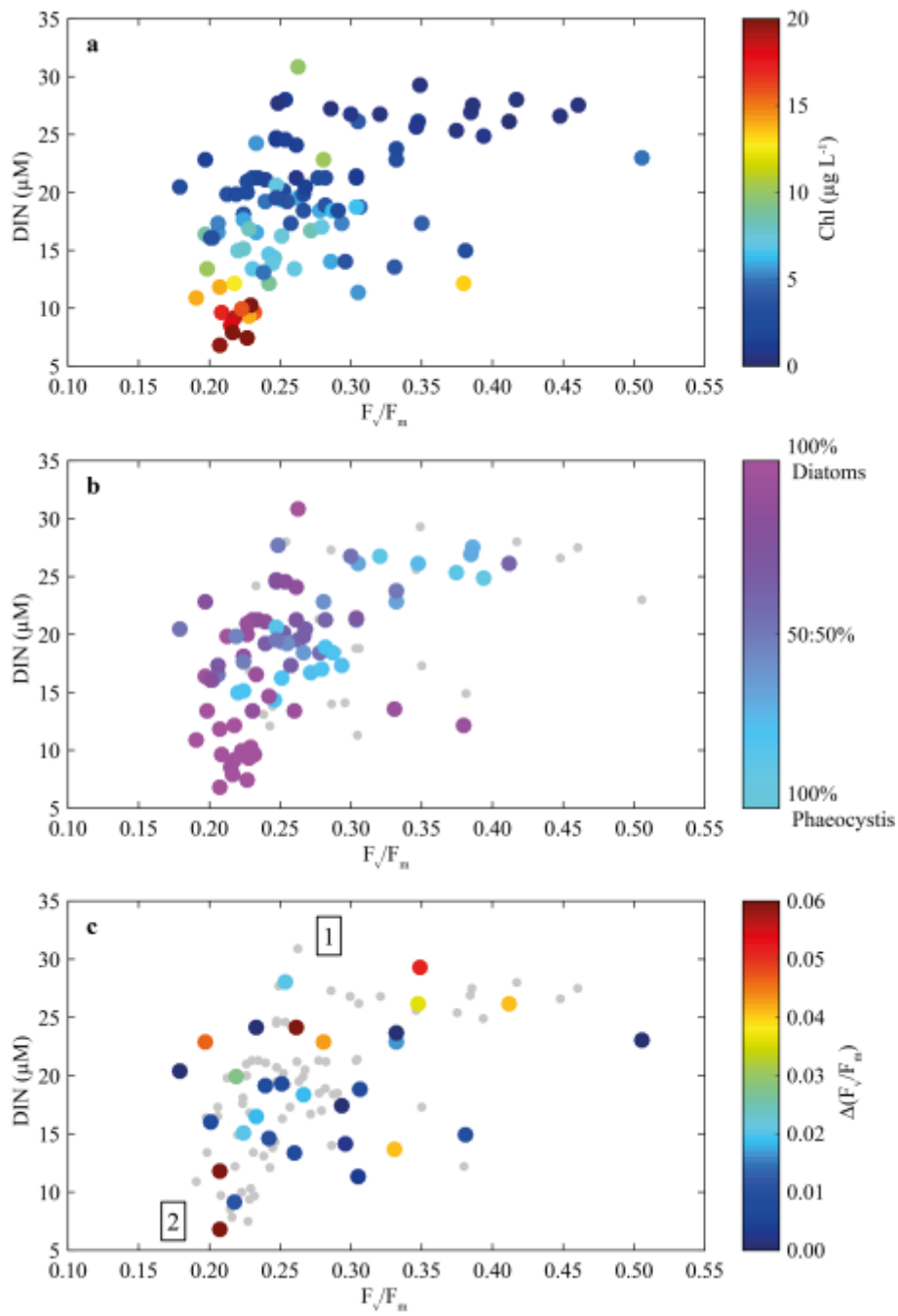


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Fig. 5

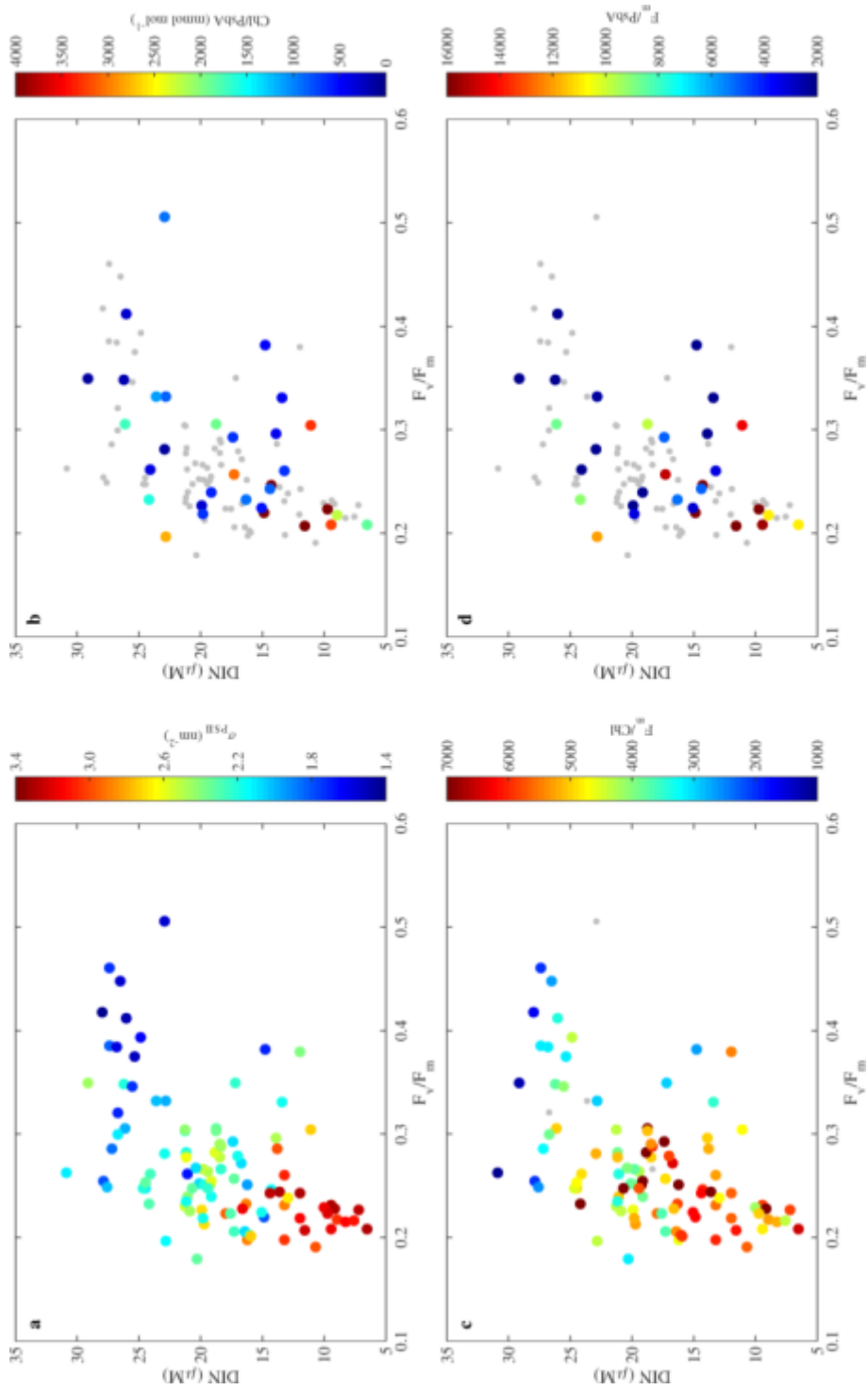


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761 Fig. 6

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Supplementary Information

Equation S1 Equation for the calculation of chlorophyll-derived net growth rates.

$$\mu^{chl} = \frac{\ln(Chl_{t=168}/Chl_{t=0})}{t}$$

where t = time in hours

Equation S2 Equation for the calculation of nitrate removal.

$$\Delta NO_3^- = \frac{[NO_3^-]_{t=168} - [NO_3^-]_{t=0}}{t}$$

Figure S1 Linear regression correlation matrices of surface variables measured in the Ross Sea. Black dots represent the data points, with black line indicating the linear regression fit with the grey shaded areas the 95% confidence interval limits.

